U.S ARMY MEDICAL SCHOOL. WASHINGTON, D.C.
DIV. OF VIRUS AND RICKETTSIAL DISEASES.

THE SEROLOGICAL PATTERN IN EPIDEMIC TYPHUS FEVER.

THE RESPIRATORY FACTOR IN EPIDEMIC TYPHUS FEVER

IV Rickettsial Localization

Harry Plotz, Colonel, MC.

Merrill J. Snyder, Sergeant.

Division of Virus and Rickettsial Diseases, Army Medical School,
Army Medical Center, Washington 12, D. C.

T 6 Dec '51

Table of Contents

The Serological Pattern in Epidemic Typhus Fever

IV. Rickettsial Agglutination.

1. Introduction.

This report is the fourth in the series on "The Serological Pattern in Epidemic Typhus Fever." The other reports dealt with "I. The Development of the Complement Fixing Antibodies", "II. The Weil-Felix Reaction", and "III. The Neutralizing Antibody."

The samples of serum used in this study represent serial serum specimens obtained from 32 cases of epidemic typhus fever studies in Cairo, Egypt. All of the antibody studies were performed on the same specimens of serum.

2. Literature.

As rickettsial suspensions became available investigators employed them to perform agglutination studies. The early workers used living rickettsiae obtained from intestinal contents of infected lice as antigen or the Weigl vaccine. By means of a microscopic technique Otto and Dietrich (1), Weigl (2), Krukowski (3), da Rocha-Lima (4), and Epstein (5) reported that convalescent epidemic typhus sera agglutinated these antigens Zinsser and Castaneda (6) likewise confirmed this finding and observed further that sera from patients convalescent from murine typhus agglutinated this antigen and that murine rickettsiae obtained from the peritoneal exudate of X-rayed rats were agglutinated by both epidemic and murine convalescent sera. Fitzpatrick and Hampil (7) prepared rickettsial suspensions from agar tissue cultures and by means of a microscopic agglutination test observed the development of agglutinins in rabbits infected with typhus.

Hudson (8) prepared murine suspensions from rat and mouse lungs infected by intranasal instillation and by means of a macroscopic test demonstrated the presence of agglutinins in rabbit and guinea pig convalescent sera as well as in six cases of human typhus. Castaneda and Silva (9) used the same type of lung suspensions to demonstrate typhus cross-agglutination in Rocky Mountain spotted fever and Castaneda (10) proposed a slide agglutination test for the diagnosis of typhus using the same antigen. Stuart-Harris, Rettie and Oliver (11), using epidemic and murine suspensions prepared from mouse lungs infected by the intranasal route, observed agglutinins in guinea pig and human convalescent specimens and concluded that rickettsial agglutination is a possible method of demonstrating differences between epidemic and murine typhus.

Van Rooyen and Bearcroft (12) used, for the first time, epidemic and murine suspensions prepared from yolk sac cultures. Using a macroscopic agglutination technique with human convalescent specimens, these authors concluded that a differential diagnosis between epidemic

The macroscopic tests described had certain disadvantages. Van Rooyen and Rhoades (12) observed that, "With the rickettsial agglutination reaction care is essential, since clumping is exceedingly fine in character and is liable to escape detection. We have found that the best method is to hold the tube up to the light and then with forefinger and thumb sharply rotate the tube twice. If agglutination is present it will be visible as a fine granular flocculum which arises into the supernatant fluid. Should difficulty be experienced in the reading of the test, the supernatant clear fluid should be discarded, a film made from the deposit of agglutinated rickettsiae, stained by Machiavello method and examined under the microscope for signs of clumping." These difficulties were overcome by using a modified technique to be described in this paper.

Even though different methods of performing the test were suggested, all observers concluded that agglutinins could be demonstrated in convalescent human and animal specimens. However, since a systematic study on serial specimens from human cases had not been carried out, there was a difference of opinion as to when this antibody makes its appearance, when it disappears, and what constitutes a significant titer. The purpose of the present study was to obtain answers to these questions insofar as our series of specimens would permit.

3. Technique.

As noted some authors have had difficulties in that aggregation has not been readily visualized and on agitation the rapid dispersal of the agglutinated rickettsiae has made reading difficult. After various attempts to devise a method to give more reproducible endpoints, it was found that the use of conical tubes promoted a greater aggregation of particles and greatly facilitated reading. While smaller tubes may be used, it has been found that the 3 cc. conical Pyrex centrifuge tubes (Corning Glass Works, Catalog No. 8060) measuring 10 mm. x 65 mm. have given optimal results.

Using these tubes, the aggregation of rickettsiae was firm and not easily dispersed. It was not necessary to centrifuge the tubes or to examine stained preparations in order to demonstrate agglutination. The test was extremely easy to read, especially when examined by artificial light. When the tests were read by two or more observers, comparable endpoints were recorded.

The suspensions employed were prepared from the Breinl (epidemic) and Wilmington (murine) strains. These agents were cultures in yolk sacs and the antigen was prepared in the identical manner as was previously described for the preparation of the purified washed rickettsial suspensions used for complement fixation (13). The suspensions were homogeneous and free of egg proteins. The antigens were standardized to a nitrogen content of 0.05 mg. N/cc. since it was found that suspensions of this density gave optimal results with the technique employed. Since the agglutination tests were performed with suspensions of the same nitrogen concentration the results were comparable (13). One technical detail of importance must be noted.

It was necessary to add normal human serum in sufficient quantity to give a concentration of 1:200 (0.5%) in the antigen preparation to be used. This small amount of serum was sufficient to prevent spontaneous agglutination of the rickettsiae.

Serial dilutions of serum were made in physiological saline and distributed in 0.25 cc. amounts. To each dilution of serum was added 0.25 cc. of rickettsial suspension. The mixture was then thoroughly shaken and placed in a water bath at 42° C. for 4 hours followed by storage in the ice box at 4° C. for another 16 to 18 hours when the test was read. A test was recorded as "complete" agglutination when the clumps had settled to the bottom of the tube, leaving a clear supernate, while a "partial" agglutination was one where definite clumping had occurred and settled out, but the supernate remained slightly cloudy. Only complete and partial agglutinations were recorded and all titers represented final dilution. Positive and negative controls were included with each test. (The test is illustrated in the accompanying photograph.)

4. Studies on control sera.

The following specimens were studied to determine the degree of agglutination obtained in diseases other than epidemic typhus fever:

a. Wassermann positive sera.

Rickettsial Agglutination with Wassermann Positive Specimens

No. of Specimens Tested	Epidemic	Murine
46	0	0
1	0	1/40
1	0	1/20

The agglutination in the two positive samples was fine, adhered to the surface of the tube and was not typical of the agglutination observed in typhus.

b. Rocky Mountain spotted fever.

Castaneda and Silva (9), using a suspension of murine rickettsiae obtained from rat lungs and convalescent human serum from cases of Rocky Mountain spotted fever, and employing a macroscopic agglutination test, observed complete agglutination in a titer of 1/160 and 1/20 and partial agglutination at 1/20 in the three specimens examined. The sera of Rocky Mountain spotted fever guinea pigs were likewise tested. No agglutinins developed from the 1st-6th day of disease while specimens obtained from the 6th-31st day showed agglutination to a variable degree. None occurred after the 32nd day.

We have likewise examined three Rocky Mountain spotted fever guinea pig pools and found murine agglutination in one pool at 1/40 and at 1/20 in the other two pools. Macroscopic agglutination tests were performed on 30 human convalescent specimens from 14 cases of Rocky Mountain spotted fever (14). Agglutination was observed in 24 specimens. Four cases showed both epidemic and murine agglutinins, eight showed murine agglutinins alone and one case showed no agglutination. In five cases there was a rise in titer when early and late specimens were compared. The titers varied from 1/20 to 1/2560.

The above results indicate that both epidemic and murine agglutination can occur in certain convalescent specimens from Rocky Mountain spotted fever in which case the titers can occasionally be high. This is not surprising since there is certain other evidence that indicates that Rocky Mountain spotted fever and typhus are immunologically related (9, 14, 15). It is important to bear these observations in mind, however, when using rickettsial agglutination as a diagnostic test.

c. Other diseases of the spotted fever group.

Three pools of convalescent guinea pig serum from Fievre Boutonneuse, South African tick bite fever and Brazilian spotted fever were examined. No epidemic or murine agglutinins were present.

d. Other diseases.

Van Rooyen and Bearcroft (12), using epidemic and murine rickettsial suspensions made from infected yolk sacs, observed two normal specimens that agglutinated epidemic and murine rickettsiae each in a dilution of 1/50. They likewise observed one specimen from a case of malaria (B.T.) that agglutinated epidemic rickettsiae in a dilution of 1/100 and two cases that agglutinated murine rickettsiae in a dilution of 1/100. One case of typhoid agglutinated epidemic rickettsiae in a dilution of 1/50 and another case agglutinated murine rickettsiae in a dilution of 1/50. There was one case each of sandfly fever and poliomyelitis that agglutinated murine rickettsiae in a dilution of 1/50. Stuart-Harris, Rettie and Oliver (11) found two normal specimens that agglutinated murine rickettsiae in a dilution of 1/40.

While no agglutination to either the epidemic or murine strain was observed in specimens obtained from guinea pigs convalescent to Fievre Boutonneuse, South African tick bite fever and Brazilian typhus, the possibility of finding agglutinins in <u>human</u> convalescent specimens from these diseases must be considered. The presence of agglutination titers of as high as 1/100 in diseases other than typhus or Rocky Mountain spotted fever must be borne in mind in interpreting the results of rickettsial agglutinations.

5. <u>Murine typhus</u>.

217

The following results are included in order to show the specificity of the rickettsial suspensions used in these tests, as well as to illustrate the type of reactions found in murine typhus. All of these sera were obtained from patients in the southern and southwestern parts of the United States where only murine typhus is known to occur:

Rickettsial Agglutination in Murine Typhus

Case No.	Day of Disease	Epidemic	Murine
1	15	1/640	1/2560
	43	1/640	1/2560
	75	1/320	1/1280
2	12	1/160	1/1280
	31	1/640	1/10,240
	47	1/320	1/2560
3	16	0	1/1280
	44	1/160	1/640
4	48	1/320	1/2560
	145	1/160	1/640
5	21	1/320	1/1280
	36	1/320	1/1280
6	13	1/320	1/2560
	90	1/160	1/640
7	24	0	0
	121	0	1/320
8	60	1/80	1/2560
9	86	1/80	1/640
	140	1/40	1/320
10	22	1/160	1/2560
	32	1/160	1/2560
	79	0	1/320
11	26	1/320	1/2560
	34	1/160	1/1280
12	29	1/320	1/5120
	52	1/160	1/2560
13	23	1/160	1/1280
	47	0	1/640
14	41	1/320	1/2560

Rickettsial Agglutination in Murine Typhus

Case No.	Day of Disease	Epidemic	Murine
15	27	1/160	1/2560
	62	0	1/640
16	65	0	1/320
17	31	1/1280	1/10,240
	52	1/320	1/10,240
	70	1/160	1/2560
18	17	1/640	1/10,240
19	42	1/320	1/5120
	81	1/160	1/2560
20	47	1/160	1/2560
21	46	1/320	1/2560
	93	1/40	1/640
22	17	1/160	1/2560
23	39	1/640	1/10,240
24	18	1/1280	1/5120
	40	1/640	1/5120

Forty-five serum samples from 24 cases of murine typhus were examined. Taking specimen for specimen it is observed that the titer obtained with the murine rickettsial antigen was always higher than was found with the epidemic rickettsial antigen. Complement fixation tests performed on the same specimens of serum showed a specific test for murine typhus.

6. Serial specimens from 32 cases of epidemic typhus fever.

Epidemic and murine rickettsial agglutination tests were performed on serial serum specimens obtained from 32 untreated and non-vaccinated cases of epidemic typhus fever studied in Cairo, Egypt. All of the specimens from a single case were examined on the same day. The results of these tests follow:

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Homologous Antigen
7	0	0
8	0	0
10	0	0
11	1/80	1/40
14	1/640	1/160
19	1/2560	1/640
21	1/5120	1/1280
25	1/10240	1/2560
30	1/10240	1/2560
35	1/10240	1/2560
36	1/5120	1/1280
37	1/5120	1/1280
115	1/160	1/40
206	1/80	1/10

CASE NO. 1345

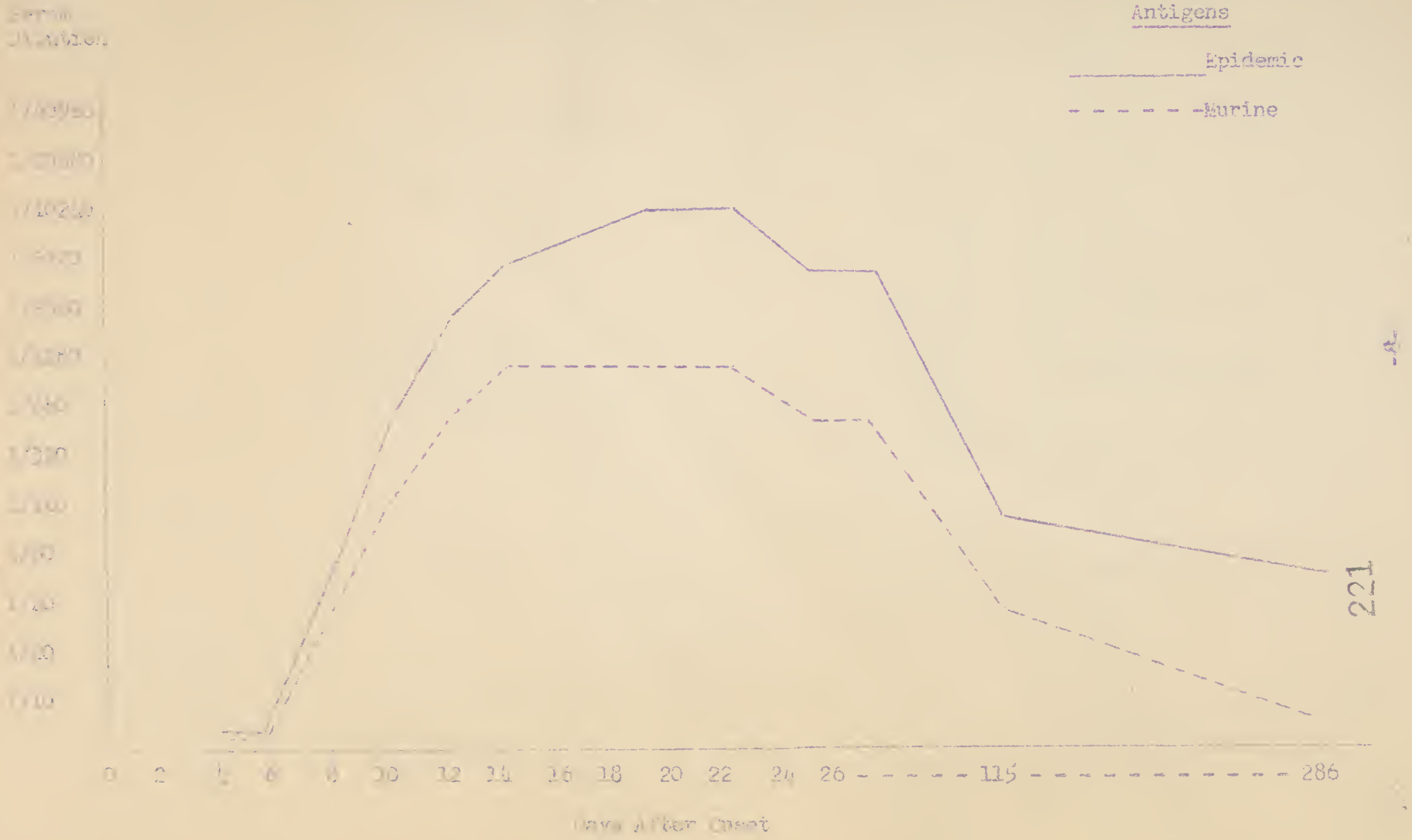
Rickettsial Agglutination

No Attempt to Isolate.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Homologous Antigen
7	1/20	0
8	1/160	1/80
10	1/640	1/320
11	1/2560	1/1280
14	1/2560	1/1280
16	1/2560	1/1280
18	1/2560	1/640
117	1/160	1/40

CASE NO. 1344

Strain Isolated



CASE NO. 1675

Rickettsial Agglutination

Strain Isolated

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
5	1/10	0
9	1/2560	1/640
11	1/10240	1/2560
13	No specimen	No specimen
17	1/10240	1/1280
19	1/10240	1/1280
24	1/5120	1/640
45	1/2560	1/160
137	1/160	1/20

CASE NO. 1690

Rickettsial Agglutination

No Attempt to Isolate.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
4	1/160	1/80
6	No specimen	No specimen
8	1/1280	1/640
10	1/2560	1/640
12	1/2560	1/1280
15	No specimen	No specimen
17	1/2560	1/1280

CASE NO. 1195

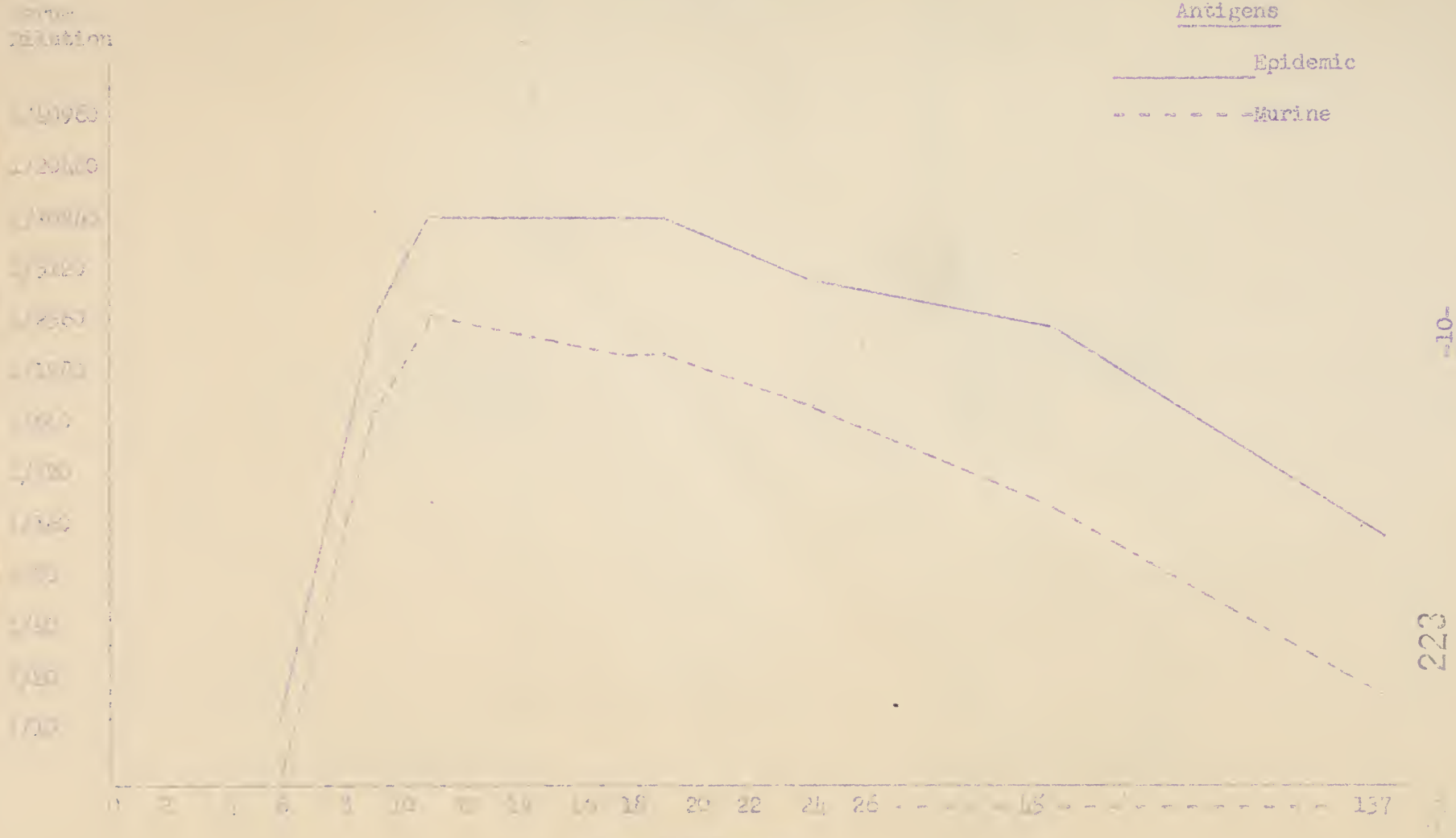
Strain Isolated

Rickettsial Agglutination

Antigens

Epidemic

Murine



-10-

223

CASE NO. 1690

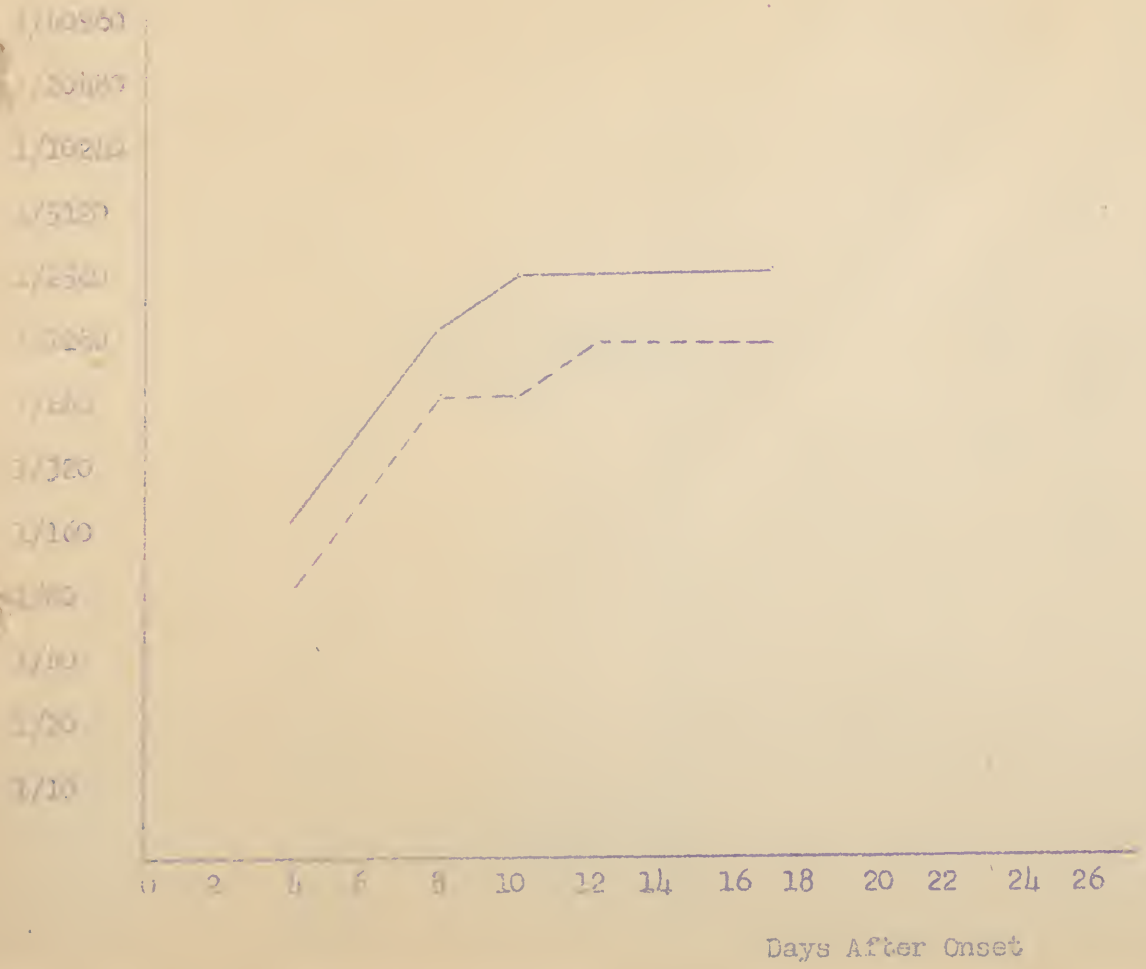
No Attempt to Isolate

Rickettsial Agglutination

Antigens

Epidemic

- - - - - -Murine



CASE NO. 1894

Rickettsial Agglutination

Strain Isolated

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
0	0	0
6	1/640	1/20
8	1/1280	1/80
11	1/2560	1/320
13	1/10240	1/1280
15	1/10240	1/1280
18	1/5120	1/1280
103	1/320	1/10
121	1/320	1/10
318	1/80	1/20

CASE NO. 1896

Rickettsial Agglutination

No Attempt to Isolate

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
5	1/160	1/80
6	No specimen	No specimen
9	1/2560	1/640
11	No specimen	No specimen
14	1/5120	1/1280
16	1/10240	1/1280
18	1/5120	1/1280
24	1/5120	1/640

CASE NO. 1902

Rickettsial Agglutination

No Attempt to Isolate.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
6	No specimen	No specimen
10	1/1280	1/160
12	1/2560	1/640
15	No specimen	No specimen
17	1/640	1/80
22	1/5120	1/640
26	1/2560	1/640
28	1/2560	1/640
32	1/2560	1/640
99	1/320	1/20

CASE NO. 2319

Rickettsial Agglutination

No Attempt to Isolate.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
7	1/2560	1/640
9	1/2560	1/640
16	1/10240	1/1280
19	1/10240	1/1280

Case No. 2271

Rickettsial Agglutination

Strain Isolated

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
6	1/320	1/80
7	1/640	1/160
8	1/2560	1/640
11	No specimen	No specimen
12	1/10240	1/1280
13	1/10240	1/1280
17	1/10240	1/1280
19	1/20480	1/2560
21	1/20480	1/2560

CASE NO. 2442

Rickettsial Agglutination

Strain Isolated

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
5	1/40	0
10	1/5120	1/320
14	1/5120	1/320
17	1/10240	1/640
21	1/10240	1/1280

CASE NO. 2723

Rickettsial Agglutination

No Attempt to Isolate.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
6	0	0
8	1/320	1/40
10	1/1280	1/160
12	1/2560	1/320
16	1/2560	1/640
20	1/2560	1/640
22	1/2560	1/320
24	1/2560	1/320
39	1/1280	1/160
88	1/640	1/80

CASE NO. 2724

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
8	1/80	0
11	1/160	1/40
13	1/1280	1/320
15	No specimen	No specimen
19	1/5120	1/1280
23	1/10240	1/1280
25	1/5120	1/1280
27	1/5120	1/640
86	1/640	1/40
286	1/80	1/80

CASE NO. 2965

Rickettsial Agglutination

No Attempt to Isolate.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
6	1/160	1/40
10	1/5120	1/1280
12	1/5120	1/1280
14	1/5120	1/1280
19	1/10240	1/2560
22	1/10240	1/2560
25	1/10240	1/2560
36	1/1280	1/160

CASE NO. 2990

Rickettsial Agglutination

No Attempt to Isolate.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
8	1/320	1/80
12	1/5120	1/320
14	1/5120	1/320
16	1/5120	1/640
19	1/5120	1/320
24	1/5120	1/320
27	1/5120	1/640
42	1/2560	1/160

CASE NO. 2980

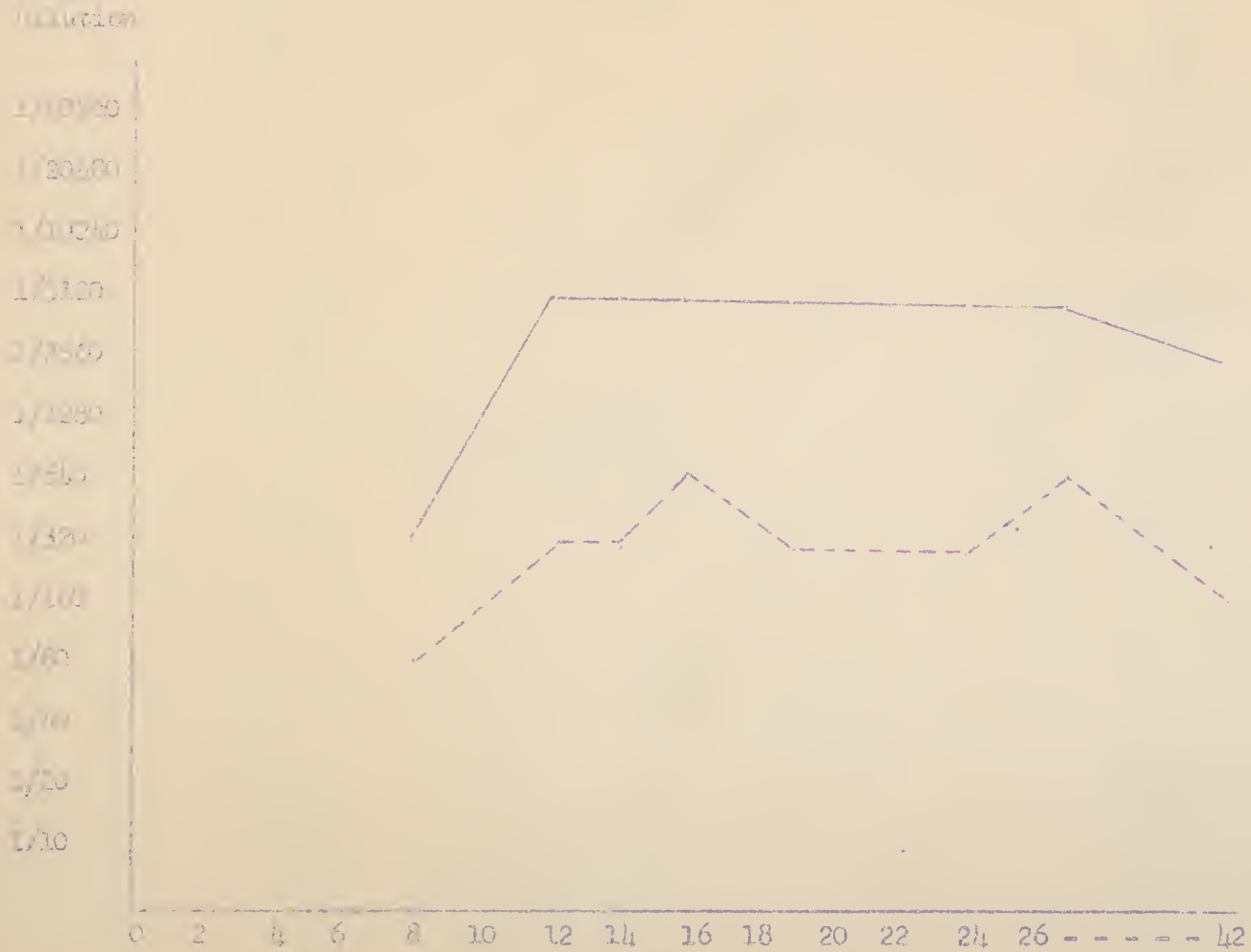
No Attempt to Isolate

Rickettsial Agglutination

Antigens

Epidemic

- - - - - - Murine



CASE NO. 3732

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
2	0	0
5	0	0
7	1/80	0
10	1/5120	1/640
13	1/10240	1/1280
16	1/10240	1/1280
18	1/10240	1/1280
20	1/20480	1/1280
34	1/2560	1/320
91	1/5120	1/320
251	1/160	1/40

CASE NO. 3732

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
3	0	0
5	1/320	1/80
8	1/5120	1/640
11	1/10240	1/640
14	1/10240	1/1280
16	1/10240	1/640
19	1/10240	1/640
90	1/320	1/80
245	1/160	1/40

CASE NO. 3732

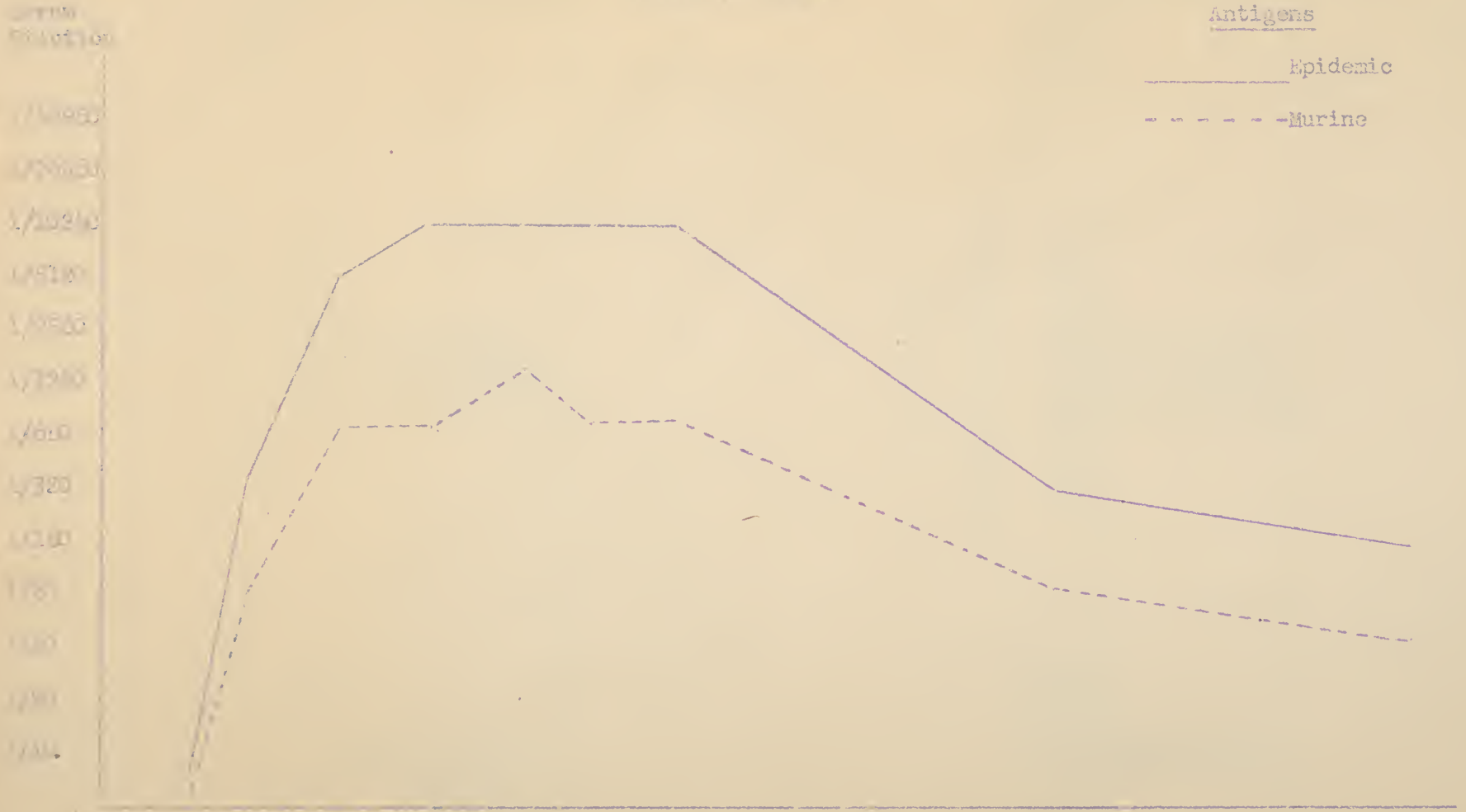
Strain Isolated

Rickettsial Agglutination

Antigens

Epidemic

- - - - - -Murine



CASE NO. 3877

Rickettsial Agglutination

Virus Isolated

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
7	1/160	1/20
8	1/320	1/80
12	1/5120	1/640
15	1/5120	1/1280
16	1/5120	1/2480
20	1/2560	1/1480
22	1/2560	1/1980

CASE NO. 3919

Rickettsial Agglutination

An Attempt to Isolate

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
6	1/160	1/20
8	1/640	1/80
11	1/1280	1/320
14	1/5120	1/640
17	1/5120	1/640
19	1/5120	1/640
21	1/5120	1/640

CASE NO. 4949

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
8	1/640	1/80
9	1/2560	1/640
11	1/10240	1/2560
14	1/5120	1/2560
16	1/10240	1/2560
17	1/10240	1/1280
19	1/10240	1/1280
23	1/10240	1/1280
64	1/1280	1/320
83	1/1280	1/320
104	1/640	1/320

CASE NO. 4956

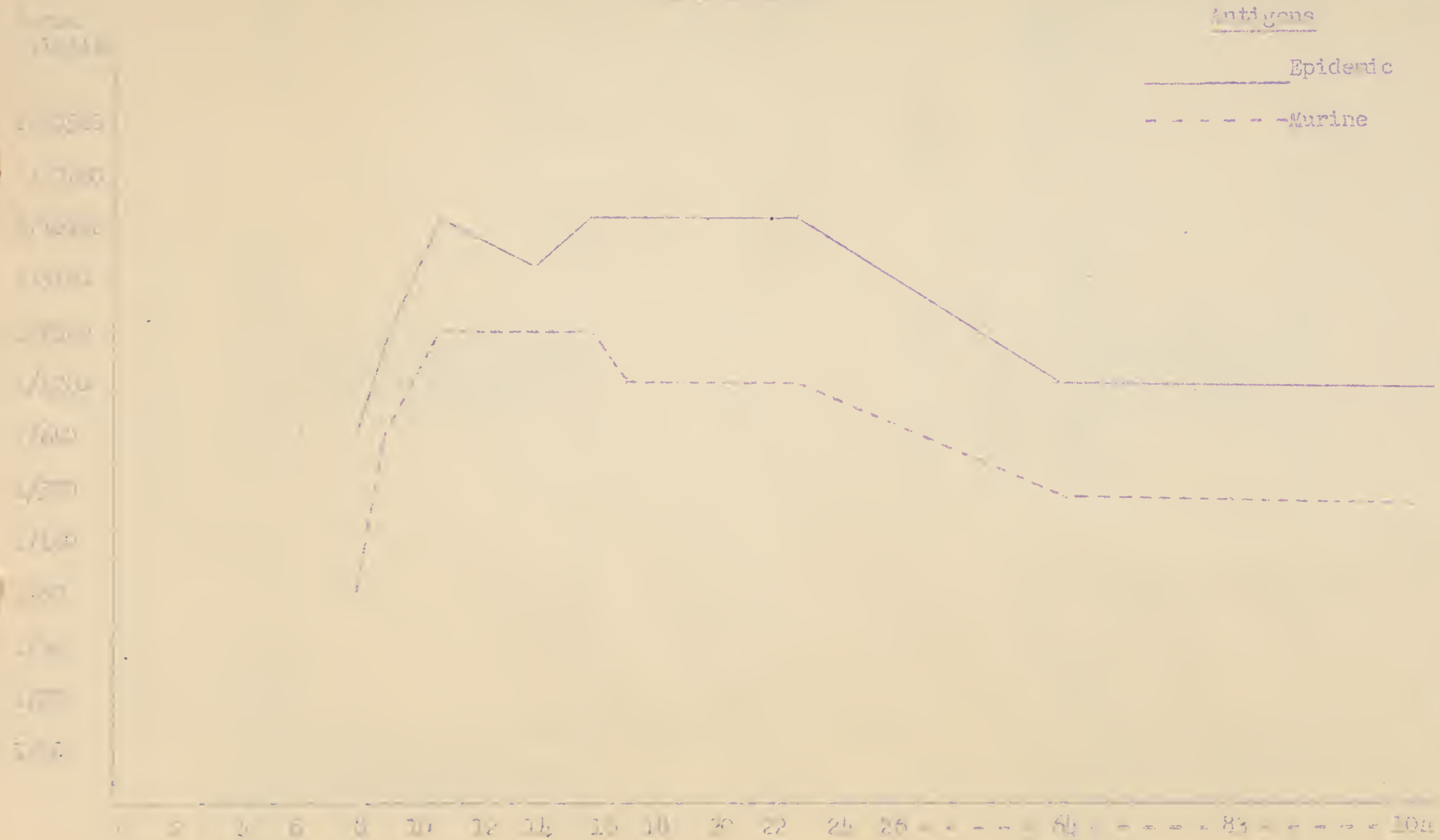
Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
8	1/160	1/20
11	1/1280	1/160
14	1/80	0
16	1/5120	1/1280
20	1/10240	1/1280
59	1/1280	1/160
86	1/640	1/80
235	1/80	1/40

CASE NO. 4569

Strain Isolated



CASE NO. 5038

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
6	1/40	0
9	1/640	1/80
12	1/5120	1/320
14	1/5120	1/320
16	1/10240	1/640
18	1/20480	1/1280
20	1/10240	1/640
22	1/5120	1/640
28	1/320	1/40

CASE NO. 5038

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
8	1/80	1/40
11	1/1280	1/160
14	1/1280	1/160
16	1/2560	1/320
18	1/2560	1/640
20	1/2560	1/320
22	1/1280	1/320
24	1/1280	1/320
41	1/320	1/80
241	1/40	1/40

CASE NO. 5585

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
6	1/20	1/10
11	1/5120	1/640
15	1/40960	1/5120
17	1/40960	1/5120
18	1/20480	1/5120
21	1/20480	1/5120
23	1/20480	1/5120
25	1/10240	1/2560
27	1/10240	1/2560
29	1/10240	1/2560
31	1/10240	1/1280
33	1/5120	1/1280
35	1/5120	1/640
37	1/1280	1/160
229	1/320	1/80

CASE NO. 5585

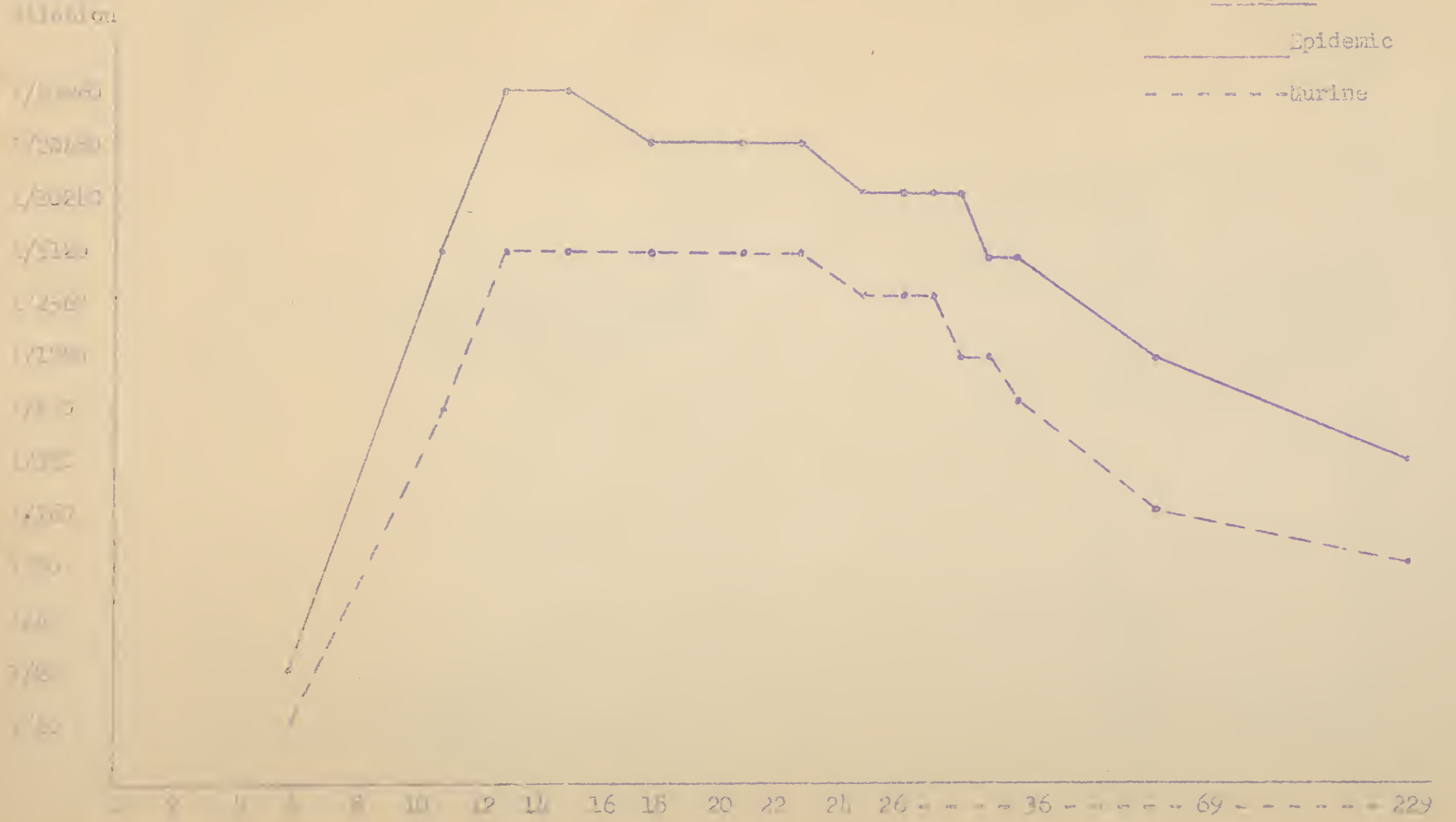
Strain Isolated

Rickettsial Agglutination

Antigens

Epidemic

Murine



CASE NO. 5706

Rickettsial Agglutination

Virus Isolated,

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
7	1/320	1/80
9	1/640	1/160
10	1/2560	1/640
12	1/2560	1/640
14	1/2560	1/640
16	1/2560	1/640
19	1/5120	1/1280
20	1/5120	1/1280
24	1/5120	1/640
25	1/2560	1/640
28	1/5120	1/640
30	1/2560	1/160
32	1/2560	1/320
34	1/2560	1/320
36	1/2560	1/160
71	1/320	1/40
226	1/160	1/10

CASE NO. 5708

Rickettsial Agglutination

Virus Isolated,

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
7	0	0
9	1/10	0
11	1/80	1/20
13	1/1280	1/160
15	1/5120	1/320
18	1/10240	1/640
21	1/10240	1/640
43	1/2560	1/160

CASE NO. 5587

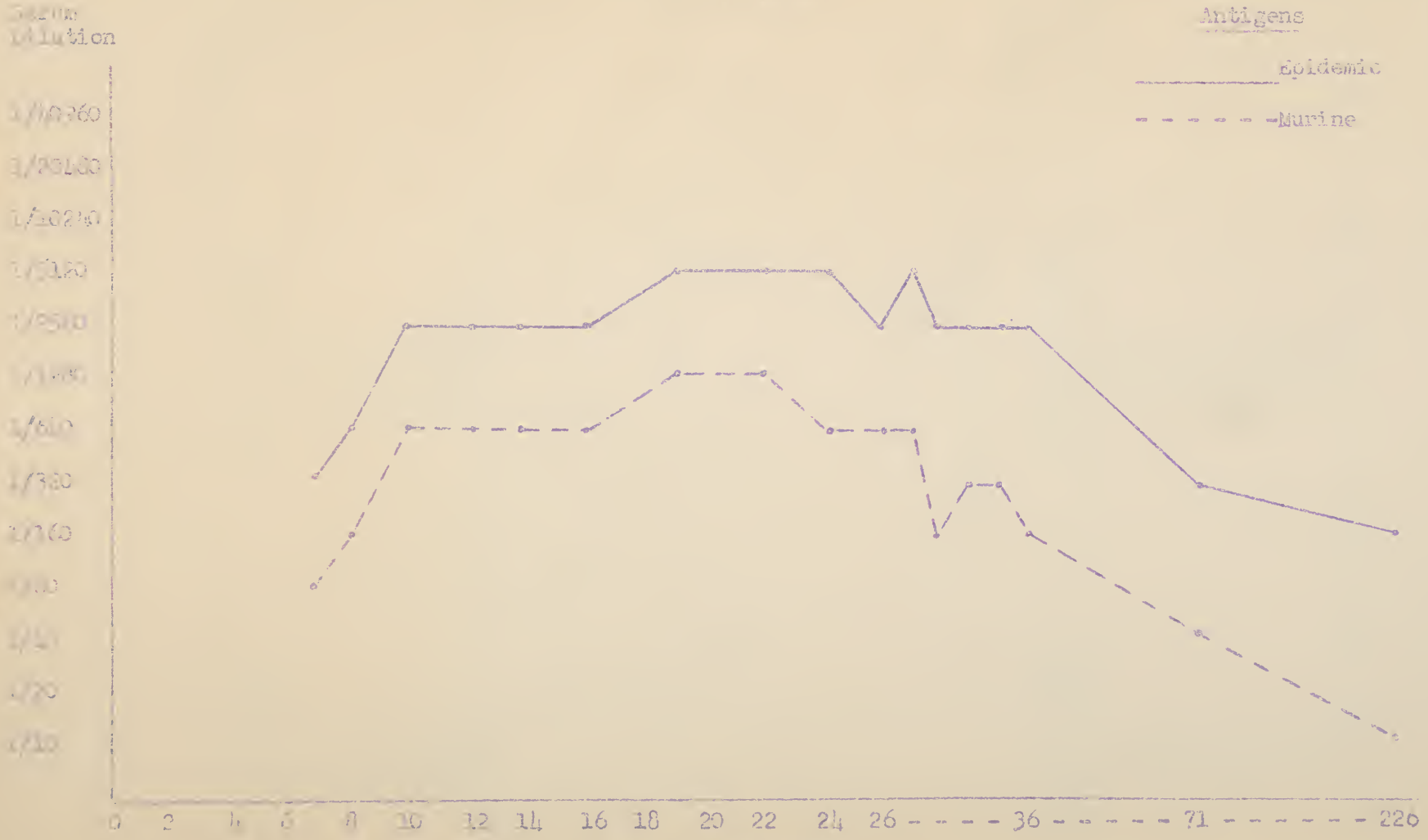
Strain Isolated

Rickettsial Agglutination

Antigens

Epidemic

- - - - - -Murine



CASE NO. 5490

Rickettsial Agglutination

Failed to Isolate Strain.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
8	1/5120	1/1280
10	1/10240	1/1280
12	1/10240	1/2560
14	1/20480	1/10240
17	1/20480	1/5120
20	1/20480	1/2560
22	1/10240	1/2560
24	1/5120	1/2560

CASE NO. 6243

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
5	0	0
8	1/160	1/40
11	1/2560	1/640
14	1/5120	1/640
16	1/5120	1/640
18	1/10240	1/2560
20	1/5120	1/640
22	1/5120	1/320
69	1/640	1/40
218	1/80	1/40

CASE NO. 6243

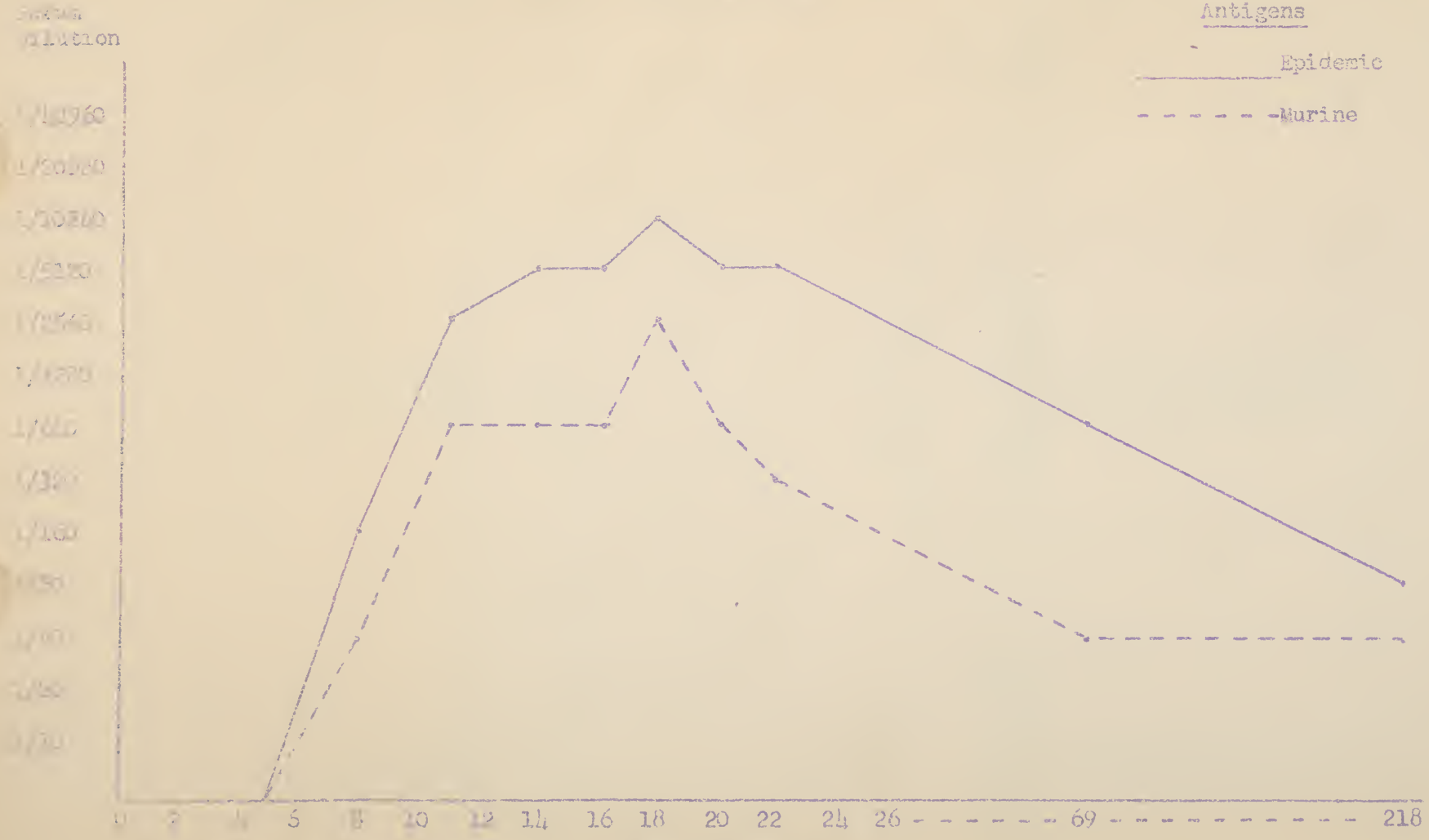
Strain Isolated

Rickettsial Agglutination

Antigens

Epidemic

Murine



CASE NO. 6953

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
8	1/1280	1/160
10	1/5120	1/640
11	1/5120	1/1280
14	1/20480	1/2560
16	1/20480	1/2560

CASE NO. 7654

Rickettsial Agglutination

Strain Isolated

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
6	0	0
8	1/20	0
10	1/320	1/40
12	1/2560	1/320
14	1/10240	1/640
16	1/5120	1/640
18	1/10240	1/640
20	1/10240	1/640
22	1/10240	1/640
24	1/10240	1/320
27	1/5120	1/320
62	1/640	1/20

243

CASE NO. 7990

Rickettsial Agglutination

Strain Isolated

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
6	1/20	0
8	1/320	1/80
10	1/2560	1/640
12	1/5120	1/1280
14	1/5120	1/2560
16	1/5120	1/2560
18	1/5120	1/1280
22	1/5120	1/1280
24	1/5120	1/1280
56	1/640	1/160
74	1/320	1/40
209	1/80	1/40

CASE NO. 8119

Rickettsial Agglutination

Strain Isolated.

Day of Disease	Serum Titer With Epidemic Antigen	Serum Titer With Endemic Antigen
7	1/10	0
8	1/40	1/20
10	1/160	1/40
12	1/2560	1/320
14	1/5120	1/640
16	1/5120	1/1280
18	1/10240	1/1280
20	1/10240	1/1280
22	1/10240	1/1280
25	1/10240	1/1280
27	1/10240	1/1280
30	1/10240	1/1280
32	1/5120	1/640

CASE NO. 7932

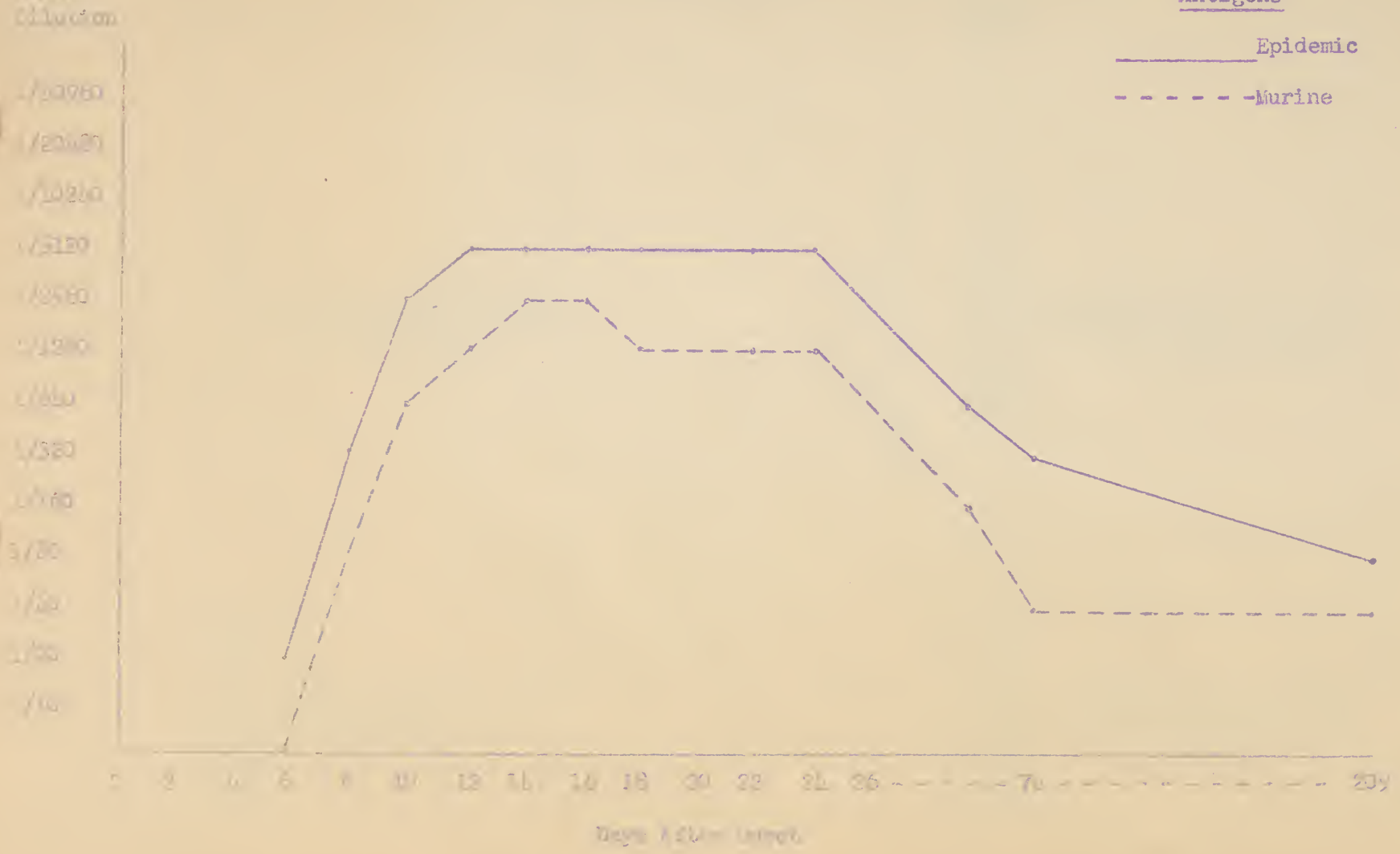
Strain Isolated

Rickettsial Agglutination

Antigens

Epidemic

- - - - - -Murine



245

7. Analysis of results.

These data can be analyzed by accepting as significant (1) any titer, (2) a titer of 1/160, or (3) a two-fold rise in titer.

If we accept any titer as being significant, then 41% of the cases would have been positive by the sixth day, 94% by the eighth day and 100% by the tenth day. If a titer of 1/160 is accepted, then 43% of the cases would have been positive by the eighth day, 71% by the tenth day and 100% by the thirteenth day. If we accept a two-fold rise in titer, then 34% of the cases would have been positive by the eighth day, 59% by the tenth day and 84% by the thirteenth day and 100% by the sixteenth day. This is shown graphically in the chart on page 34.

Since titers of as high as 1/100 have been found in diseases other than typhus, it is considered safer to accept titers higher than 1/100 as being significant. As with the Weil-Felix test, it is considered advisable, however, to accept a rise in antibody titer rather than the results obtained from a single specimen.

It is observed that rickettsial agglutinins occurred in all cases and that a rise in titer followed when early and late specimens were compared. The titer with the epidemic antigen always exceeded that found with the murine antigen even though frequently there was only a one tube difference. Titers with the epidemic rickettsial antigen occasionally reached a dilution of 1/20,480. Cross-agglutination was the rule.

8. Persistence of rickettsial agglutinins.

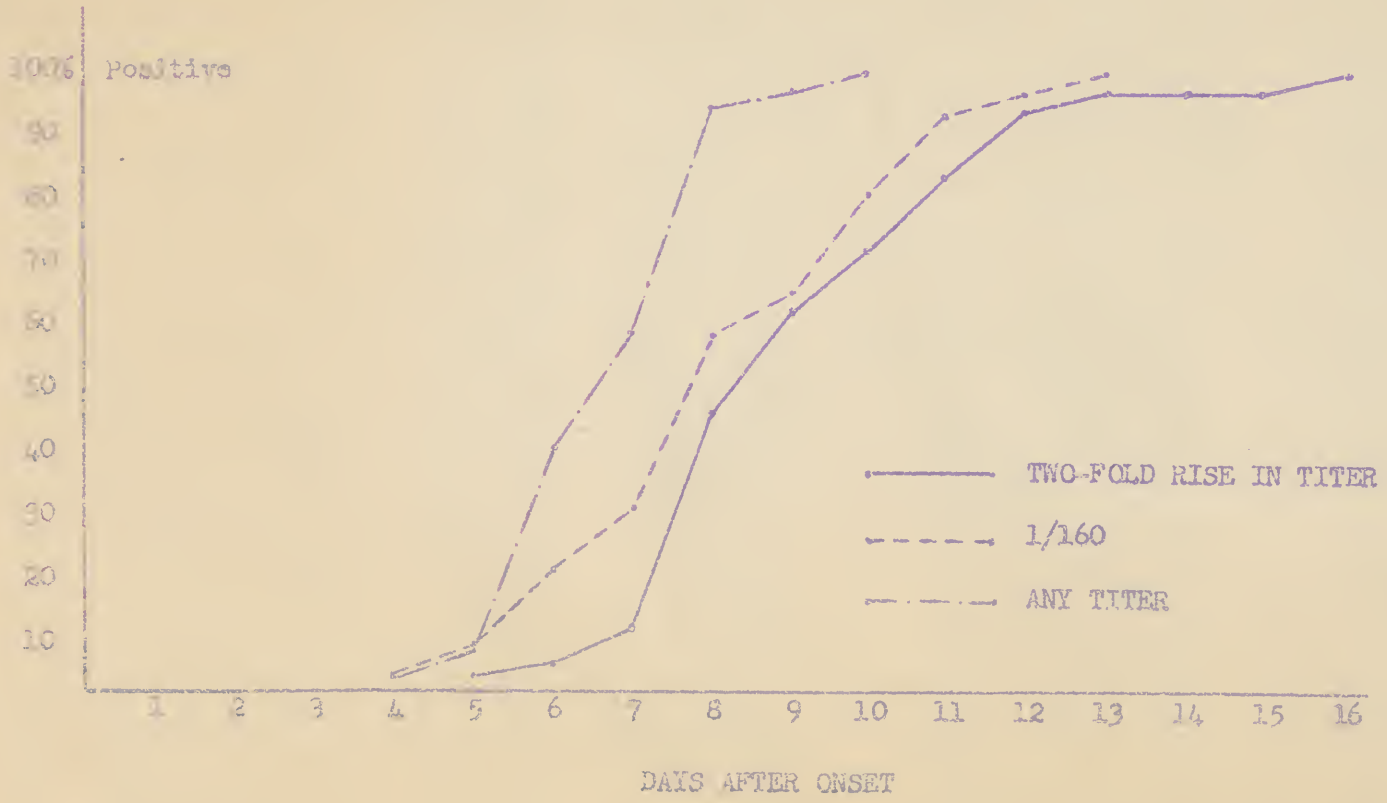
Since the demonstration of the presence of specific antibodies may be used as an aid in diagnosis or as an indication of the presence of a past infection, it is of value to examine these data as to the persistence of the agglutinating antibody in epidemic typhus fever. Within the limits of this study it is found that rickettsial agglutination had reached a titer below the significant level of 1/160 in 37% of the cases studied by three months after onset of the disease. All cases except three had fallen below this titer after 10 months. In these three cases, titers of 1/640, 1/320 and 1/320 were observed after 104, 229 and 276 days, respectively.

9. Summary.

A standardized rickettsial agglutination test is described which is easy to perform and read. Epidemic and murine rickettsial agglutinations were performed on serial specimens from 32 cases of epidemic typhus fever and the results are recorded. It is seen that agglutinins occur in rising titer in all cases some time or other during the course of the disease. Since the titer obtained with the epidemic antigen exceeds that found with the murine antigen, a differentiation between these two types of typhus can be made by means of this test.

RICKETTSIAL AGGLUTINATION

RICKETTSIA PROWAZEKI



It appears that this antibody reaches a level below a diagnostic titer in late convalescence and hence this test cannot be used to demonstrate the existence of a past infection. Since titers of 1/100 are occasionally found in unrelated diseases, it would be safer to accept higher titers as being diagnostically significant. Since epidemic and murine agglutinins may occur in convalescent specimens from cases of Rocky Mountain spotted fever, occasionally in high titer, caution should be observed in evaluating this test when used as a diagnostic procedure.

1. Otto, R. and Dietrich, A., Deutsch. Med. Woch., 1917, 43, 577.

2. Weigl, R., Zschr. f. Hyg., 1923, 99, 302.

3. a. Krukowski, Medycynij doswiadczalnej spolecznej, 1923, 1, 378.
   b. Krukowski, Medycynij doswiadczalnej spolecznej, 1924, 2, 370.

4. da Rocha-Lima, H., cited from Otto, R. and Munter, H., in Handbuch der Pathogenen Microorganismen, Kolle, W., Kraus, R., and Uhlenhuth, P., editors, 3rd edition, 1930, Band 8, Liefg. 44, 1193.

5. Epstein, H., Zentralft. f. Bact., 1921-22, 87, 553.

6. Zinsser, H. and Castaneda, M. R., J. Exp. Med., 1932, 56, 455.

7. Fitzpatrick, F. and Hampil, B., Amer. Jour. Pub. Health, 1941, 31, 301.

8. Hudson, P., Jour. Infec. Dis., 1940, 67, 227.

9. Castaneda, M. R. and Silva, Jour. Immun., 1941, 42, 1.

10. Castaneda, M. R., Puerto Rico Jour. of Pub. Health and Trop. Med., 1942, 18, 165.

11. Stuart-Harris, C. H., Rettie, G. K. C., and Oliver, J. C., Lancet, 1943, 2, 537.

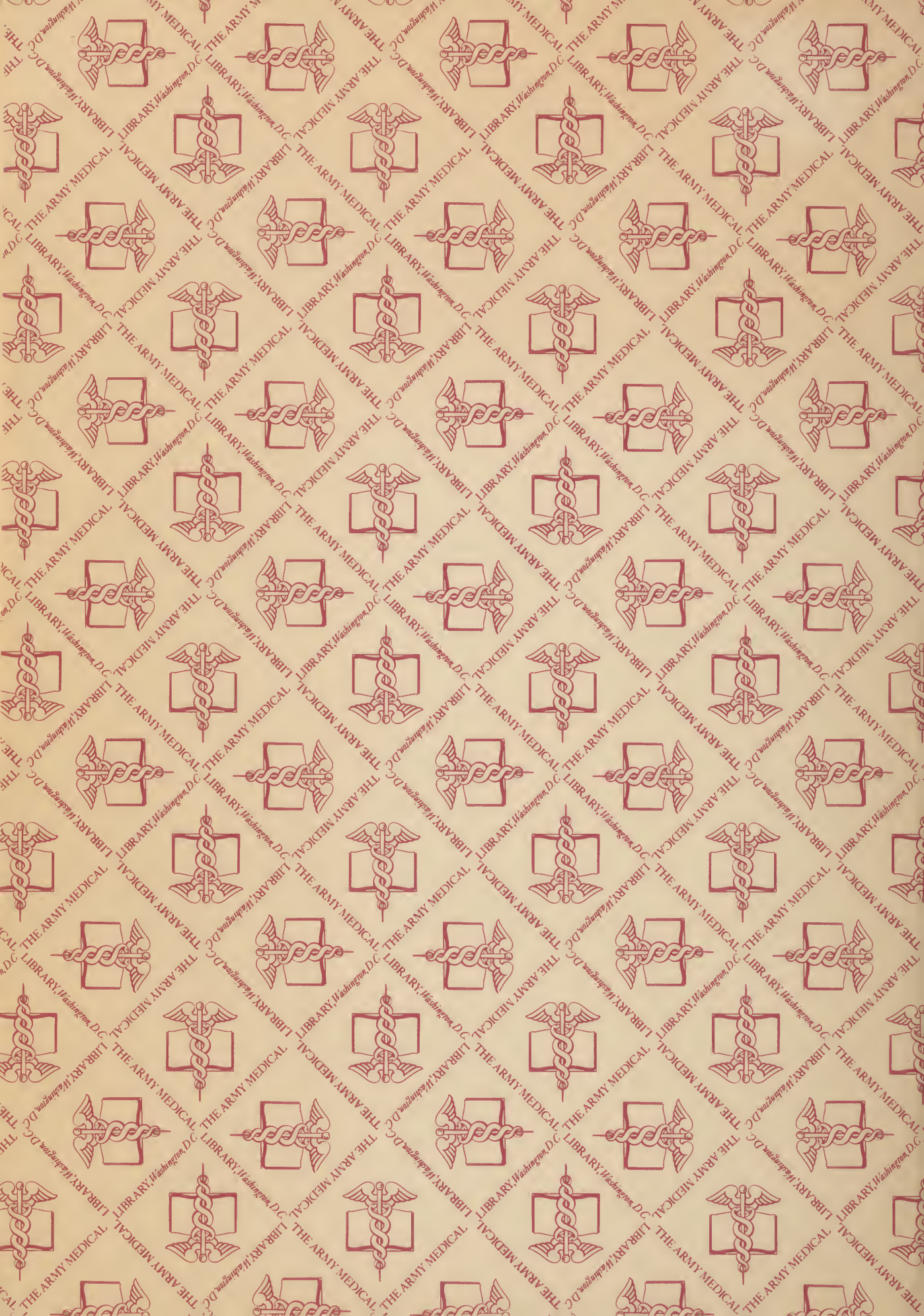
12. Van Rooyen, C. E., and Bearcroft, M. C., Edin. Med. Jour., 1943, 50, 257.

13. Plotz, H., Wertman, K., and Bennett, B., Report to the Surgeon General (withheld from publication).

14. Plotz, H., Bennett, B., Wertman, K., and Snyder, M., to be published.

15. Parker, R. R., Public Health Reports, 1943, 58, 721.

249


THE ARMY MEDICAL LIBRARY, Washington, D.C.